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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 9/28, 39/15, 39/175, 39/215, 39/23

A1

(11) International Publication Number: **WO 98/18453**

(43) International Publication Date: 7 May 1998 (07.05.98)

(21) International Application Number: PCT/IB97/01136

(22) International Filing Date: 22 September 1997 (22.09.97)

(30) Priority Data:
60/028,802 28 October 1996 (28.10.96) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ORAL VACCINES FOR YOUNG ANIMALS WITH AN ENTERIC COATING

(57) Abstract

The present invention provides a vaccine formulation and method for oral vaccination of animals of weaning age or younger against a pathogen in the presence of interfering maternal antibodies. The formulation of the invention comprises an antigen in an enteric coating.

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ORAL VACCINES FOR YOUNG ANIMALS WITH AN ENTERIC COATING

FIELD OF THE INVENTION

The present invention relates to compositions and methods for oral vaccination of animals of weaning age or younger against disease.

BACKGROUND OF THE INVENTION

Vaccination of animals of weaning age or younger against a disease-causing pathogen is often compromised by the presence of interfering maternal antibodies, *i.e.*, maternally-derived antibodies that are directed against the pathogen. For example, canine parvovirus (CPV), canine distemper virus (CDV), canine coronavirus (CCV) and canine rotavirus (CRV) diseases are the most important diseases in dogs of weaning-age or younger. However, vaccination of these young dogs against these viral pathogens is compromised by the presence of the interfering maternal antibodies. This creates a "window of susceptibility" to infection in the young animal because the maternal antibody titer in the animal is often high enough to interfere with vaccination, but too low to provide an adequate level of protection. Parenterally-injected vaccines are of less than optimal efficacy in these young animals, and must generally be administered multiple times to ensure adequate immunity. In addition, parenterally-injected vaccines often do not induce immunity until at least several weeks after weaning, depending upon the titer of the interfering maternal antibodies.

Since the entry points for many pathogens, including CPV, CDV, CCV and CRV, are through the oral and nasal routes, oral immunization may trigger a more rapid and robust immune response against one or more of these pathogens by eliciting the production of secretory immunoglobulin A (IgA) antibodies at mucosal sites. Such an immune response is triggered when an antigen is presented to the epithelium of gut-associated lymphoid tissues, such as Peyer's patches. See, for example, O'Hagan, 1992, Clin. Pharmacokinet. 22: 1-10; Caldwell *et al.*, 1982, J. Pharm. Pharmacol. 34:520; and U.S. Pat. No. 5,075,109. However, oral immunization has generally remained underexploited as a method of vaccination because major problems remain over how to deliver an oral vaccine to the gut

mucosal tissue without significant degradation in the digestive tract (O'Hagan, 1992, above).

Compositions and methods have been described for the oral immunization of humans and various animal species. For example, U.S. Pat. No. 5,352,448 discloses an oral vaccine formulation for ruminants, comprising an antigen composition in a delivery vehicle consisting of a water-swellable hydrogel matrix, which allows for delivery of the antigen to mucosa-associated lymphoid tissue in the post-ruminal portion of the digestive tract. U.S. Pat. No. 5,176,909 discloses compositions for oral administration to humans or animals, comprising an immunogen, gelatin of a particular molecular weight range, and an enteric coating. U.S. Pat. No. 5,075,109 discloses a method for targeting a bioactive agent, *e.g.*, an antigen, to the Peyer's patches by microencapsulating the agent in a biocompatible polymer or copolymer, such as poly(DL-lactide-co-glycolide). U.S. Pat. No. 5,032,405 discloses an oral formulation, comprising a lyophilized mixture of a biologically active agent, *e.g.*, an immunogen, in combination with maltose, a particulate diluent, and a coating comprising an alkaline-soluble polymeric film. U.S. Pat. No. 4,152,415 discloses a method of immunizing field-raised swine against dysentery, comprising administering a sequential series of parenteral and enteric-coated oral preparations of a virulent isolate of killed cells of *Treponema hyodysenteriae*.

Despite these disclosures, it is unclear whether an oral vaccine formulation can trigger a sufficiently robust immune response in animals of weaning-age or younger to overcome the presence of interfering maternal antibodies.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides an oral vaccine formulation for administration to an animal of weaning age or younger, which comprises an immunologically effective amount of an antigen in an enteric coating, and that it is capable of triggering a protective immune response against a pathogen in the presence of interfering maternal antibodies. The protective immune response can be triggered in animals at a younger age than can be obtained with conventional parenteral vaccines.

In a second aspect, the invention provides a method for vaccinating an animal of weaning age or younger against a disease, comprising orally administering to the animal a vaccine comprising an immunologically effective amount of an antigen in an enteric coating, which vaccine is capable of triggering a protective immune response against the disease, even in the presence of interfering maternal antibodies. In a non-limiting embodiment, the animal is a dog of weaning age or younger, although the invention is generally applicable to other animals of weaning age or younger, including cats, cows, pigs, sheep, and horses, among others.

Other features and advantages of the invention will be described in the following sections of the specification.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an oral vaccine against a pathogen for administration to an animal of weaning age or younger, which vaccine comprises an immunologically effective amount of an antigen prepared from the pathogen in an enteric coating, that it is capable of triggering a protective immune response against the pathogen in the presence of interfering maternal antibodies. The vaccine may be administered to any animal of weaning age or younger of a particular species, whether or not interfering maternal antibodies are present. The vaccine of the invention may be formulated for administration to any one of various species of animals, including dogs, cats, cows, pigs, sheep, and horses, among others. In a preferred but non-limiting embodiment, the animal is a dog of weaning age or younger. In a further non-limiting embodiment, the pathogen is a canine virus.

The enteric coating protects the antigen from the low pH environment of the stomach, but dissolves and releases the antigen in the region of gut-associated lymphoid tissues. As a result, a gut mucosal immune response is triggered in the animal that is sufficiently robust to overcome the interference of maternal antibodies and protect the animal against disease caused by the pathogen.

The vaccine of the invention may comprise an antigen prepared from any pathogen to which the animal is susceptible. In a non-limiting embodiment, the antigen is prepared from a viral pathogen. For example, for dogs, the antigen may be prepared from CRV, CPV, CDV or CCV. However, antigen may alternatively be

prepared from other pathogenic viruses, or from pathogenic bacteria, protozoans, or fungi that cause disease in the particular animal species. Vaccine antigens which are currently administered parenterally to the animal species, as known in the art, may be also used in the oral vaccine formulation.

For oral vaccination of a feline species, the antigen may be prepared from any pathogen that causes disease in cats including, for example, calici virus, feline herpes virus, leucopenia virus, feline coronavirus including feline infectious peritonitis (FIP) virus, non-FIP coronavirus, and rabies virus, among others.

For oral vaccination of a bovine species, the antigen may be prepared from any pathogen that causes disease in cows including, for example, bovine herpes virus, bovine respiratory syncitial virus, bovine diarrhea virus, parainfluenza type 3 virus, bovine coronavirus, bovine rotavirus, rabies virus, and *E. coli*, among others.

For oral vaccination of a swine species, the antigen may be prepared from any pathogen that causes disease in pigs, including, for example, swine parvovirus, swine pseudorabies virus, rabies virus, transmissible gastroenteritis virus, swine rotavirus, and virulent *E. coli*, among others.

The antigen may comprise any component of a viral or cellular pathogen, which component is capable of triggering a protective immune response in the animal in the presence of interfering maternal antibodies. Such a component includes, but is not limited to, whole viral particles or cells, viral capsids and envelopes, whole membrane preparations and membrane fractions, proteins, peptides, glycoproteins, exotoxins, endotoxins, enzymes, and antigenic subunits of any of the aforementioned components. Also included is a DNA sequence which encodes an antigenic peptide or protein. Smaller antigenic components may be derived from larger components such as membranes and proteins by fractionation or degradation techniques known in the art. Once obtained, the antigen may be modified using standard techniques, for example, by adding or substituting chemical substituents that alter rates of degradation *in vivo* or that increase antigenicity. The antigen may also be further processed, such as by lyophilization, according to standard techniques.

Pathogenic strains of viral particles or cells for use to prepare antigen can be obtained using standard isolation techniques from fluids, tissues or organs of infected animals exhibiting clinical symptoms of a particular disease. Alternative

sources of pathogen include publicly available deposits, such as those stored at the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852-1776, USA.

Where whole live viral particles or cells of a pathogen are used as antigen, they are preferably modified to an attenuated form, or are inactivated. Methods of attenuating a pathogen are known in the art and include, for example, serial passaging of the pathogen. For example, viral pathogens may be serially passaged in an *in vitro* culture of susceptible cells, preferably mammalian cells, and most preferably in cells of the particular animal species to be vaccinated. For example, a canine viral pathogen can be serially cultured in canine kidney cells according to known techniques, such as those described in Appel *et al.*, 1979, Vet. Records 105:156-159; U.S. Pat. No. 4,193,991; and U.S. Pat. No. 4,303,645, which are incorporated herein by reference. Other methods of attenuation include, but are not limited to, exposure of a pathogen to a mutagenic agent, *e.g.*, a chemical mutagen or ultraviolet radiation. A pathogen may also be attenuated by knocking out one or more essential metabolic genes, for example, by use of recombinant DNA techniques which are known in the art, including by homologous recombination. Alternatively, a pathogen can be inactivated, such as by exposure to formaldehyde, glutaraldehyde or ultraviolet light, among other inactivating agents.

After the attenuation step, viral particles or cells exhibiting sufficient attenuation of pathogenicity compared to the parental strain are preferably selected and clonally propagated according to known tissue culture techniques. Attenuation may be indicated, for example, by the appearance of a novel temperature sensitivity or a novel auxotrophy, or a reduction in a virulence trait, such as infectivity or the severity or rate of progression of a symptom or condition associated with the disease, among other indications. For example, attenuation of a pathogen may be indicated by the appearance of a novel temperature sensitivity in which the attenuated cells will not grow, or will grow only at a significantly reduced rate compared to the pathogenic parental strain, at the normal body temperature of the animal to be vaccinated.

The oral vaccine formulation of the invention comprises an immunologically effective amount of antigen, which is that amount of antigen capable of triggering a protective immune response in an animal of weaning age or younger in the

presence of interfering maternal antibodies. As used herein, a "protective immune response" is defined as any immunological response, based either on antibody or cell-mediated immunity, or both, occurring in the animal after administration of the vaccine of the invention, which response either prevents or reduces infection by the pathogen, or eliminates or reduces the severity, or slows the rate of progression, of one or more clinical symptoms or conditions normally associated with a disease caused by the pathogen. As used herein, "interfering maternal antibodies" refer to those antibodies present in the young animal that originate from a maternal source, are directed against a pathogen, and are present in sufficient titer to otherwise detectably reduce, or interfere with, the efficacy of a parenterally administered vaccine directed against the pathogen. For example, a titer of interfering maternal antibodies of about 1:16 or higher will interfere with successful immunization.

The oral vaccine of the invention is formulated so that the antigen is protected from degradation in the low pH environment of the stomach, thus allowing the antigen to be delivered to the region of the gut necessary to stimulate a mucosal immune response that is sufficient to trigger a protective immune response, even in the presence of interfering maternal antibodies. To accomplish this, the oral vaccine formulation comprises an immunologically effective amount of antigen protected within an enteric coating that resists dissolution in the low pH environment of the stomach, but which readily dissolves to release the antigen only at about pH 6 or higher. The enteric coating is any type of substance useful to coat the antigen that meets these requirements. Materials and methods to produce enteric coatings are known in the art. For example, a material useful to produce an enteric coating may be a polymethacrylic acid copolymer, one non-limiting example of which is a partially methylated polymethacrylic acid copolymer such as EUDRAGIT S100® type B, NF or EUDRAGIT L100® (Rohm Tech Inc., Malden, MA.). The pH at which the enteric coating dissolves can be specifically adjusted or broadened as needed, for example, by adjusting the concentration of the enteric coating component applied, or by combining one or more enteric coating components together. For example, EUDRAGIT S100® may be combined in varying ratios with EUDRAGIT L® to produce a coating that dissolves at a specifically modified pH. Such a technique is described in U.S. Pat. No. 5,032,405, which is incorporated herein by reference. The

thickness of the enteric coating may also be adjusted to help control the timing and location of dissolution of the formulation.

The oral vaccine formulation of the invention may further comprise one or more veterinarily-acceptable excipients known in the art, such as those that improve preparation, handling and stability of the formulation. Non-limiting examples of such excipients include, for example, lactose, sucrose, maltose, mannitol, starch, gums, gelatin, cellulose derivatives, magnesium salts such as magnesium stearate, magnesium carbonate, and magnesium oxide, calcium salts such as calcium carbonate and calcium sulfate, silica gel, and talc, among others. The oral vaccine formulation may optionally comprise other useful components, including, for example, an undercoating, or a colorful dye.

One non-limiting example of an oral vaccine formulation comprises the following components: antigen, lactose, magnesium stearate, modified cellulose gum, an undercoating, and an enteric coating. Various modifications of this formulation will be immediately apparent to those skilled in the fields of immunology, veterinary medicine, delivery system formulations, *etc.* Suitable other vehicles and additives are known, or will be apparent to those of skill in the art. See, *e.g.*, Remington's Pharmaceutical Science, 18th Ed., 1990, Mack Publishing, which is incorporated herein by reference.

The ingredients of the vaccine are combined according to known techniques to produce an orally administrable formulation. Thus, after preparing and mixing the antigen and any excipients, the mixture is compressed to an administrable form such as a tablet, pill, or capsule. For example, the formulation may be compressed using a Carver Lab Press (Fred. S. Carver Inc., Menomee Falls, WI.). The formulation is then provided with an enteric coating such as by the Open-Pan Ladle Coating Process or the Wurster Coating Process, which are well-known in the art.

The present invention further provides a method for vaccinating animals of weaning age or younger against a pathogen, comprising orally administering to the animal a vaccine capable of triggering a protective immune response against the pathogen, even in the presence of interfering maternal antibodies, said vaccine comprising an immunologically effective amount of antigen in an enteric coating that dissolves only at about pH 6.0 or higher. In a preferred, but non-limiting, embodiment, the vaccine formulation is administered to a dog of weaning age or

younger to protect against canine disease. The canine disease may be caused by any pathogen that infects dogs, including but not limited to CRV, CPV, CDV or CCV.

An effective dosage of the oral vaccine formulation may be determined by conventional means, starting with a low dose of the formulation and then increasing the dosage while monitoring the effects, and systematically varying the dose as well. Numerous factors may be taken into consideration when determining an optimal dose per animal. Primary among these is the breed, size, age, and general condition of the animal to be vaccinated. Other considerations include the presence and titer of interfering maternal antibodies in the animal, the presence of other drugs, and the like. The actual dose is preferably chosen after consideration of the results of other animal studies.

In a preferred embodiment, an immunologically effective amount of vaccine comprising a viral pathogen will comprise from about 10^3 to about 10^9 $TCID_{50}$ per animal; more preferably the amount will be from about 10^4 to about 10^8 $TCID_{50}$ per animal; and most preferably the amount will be from about $10^{5.5}$ to about 10^7 $TCID_{50}$ per animal. In a preferred embodiment, an effective dose range of bacteria will comprise from about 10^5 to about 10^{12} plaque-forming units (pfu) per animal.

Vaccine regimens may also be selected based on the above-described factors. Thus, animals may be vaccinated soon after birth, or just prior to, at the time of, or soon after weaning, depending upon analysis of these factors. Supplemental administrations, or boosters, may be required for full protection. One method of detecting whether adequate immune protection has been achieved is to determine seroconversion and antibody titer in the young animal after vaccination.

A benefit of the vaccine of the present invention is that a protective immune response can be triggered in animals at a younger age than can generally be obtained with conventional parenteral vaccines. For example, in dogs, whereas conventional parenteral vaccines often do not induce immunity prior to 12 to 16 weeks of age depending on maternal antibody titer, the oral vaccine of the invention can induce immunity at 4-6 weeks of age. Thus, in dogs, a preferred, though non-limiting, administration schedule is a single oral dose of the vaccine at the time of weaning, *i.e.*, at approximately 4 to 8 weeks of age. Alternatively, two oral doses may be necessary if an adequate immune response does not occur in a dog after

an initial dose, or if the dog has an interfering maternal antibody titer in excess of about 1/128.

The present invention further provides combination vaccines, comprising an immunologically effective amount of a first antigen capable of triggering a protective immune response against a pathogen in the presence of maternal antibodies, and an immunologically effective amount of one or more other antigens that can trigger a protective immune response against the same or a difference pathogen.

The following specific embodiments of the invention are intended to be illustrative and not limiting.

EXAMPLE 1: ORAL VACCINE PREPARATION

A general oral formulation was prepared as follows. A ratio by weight of 60 parts lactose (direct compression grade) (Sheffield Products, Norwich, N.Y.); I part AC-DI-SOL® modified cellulose gum (FMC Corp., Philadelphia, PA.); 1 part magnesium stearate (impalpable powder) (Mallinckrodt, St. Louis, MO.); and 4 parts lyophilized canine parvovirus that had been attenuated by serial passage in dog kidney cells, were used to make a tablet formulation. The lactose, modified cellulose gum, and lyophilized vaccine were mixed together. The magnesium stearate was added to produce a final mixture, which was compressed into tablets using a Carver Lab Press (Fred. S. Carver Inc., Menomee Falls, WI.) with a one-half inch tooling dye at 5,000 pounds/sq. inch. The tablets were rotated in a pan coater during application of OPADRY CLEAR™ undercoating (Colorcon, West Point, PA.). An enteric coating was then added by applying EUDRAGIT S100® methacrylic acid copolymer type B, NF (Rohm Tech Inc., Malden, MA.) to a final concentration of about 3% by weight using the Open-Pan Ladle Coating Process.

EXAMPLE 2: EFFICACY TESTING OF AN ORAL FORMULATION WITHOUT AN ENTERIC COATING

Attenuated CPV, CCV and CRV were grown separately in cultures of canine kidney cells to titers of $10^{6.7}$ $TCID_{50}$ of CPV, $10^{5.3}$ $TCID_{50}$ of CCV, and $10^{8.2}$ $TCID_{50}$ of CRV, respectively, according to standard tissue culture techniques. The attenuated viral particles were lyophilized and formulated together into tablets comprising $10^{6.3}$

$TCID_{50}$ of CPV, $10^{4.3}$ $TCID_{50}$ of CCV, and $10^{7.5}$ $TCID_{50}$ of CRV, and containing all of the components listed above in Example 1, but without an enteric coating.

Four 3.5- to 5.5-month-old SPF beagles, which were seronegative for anti-CPV antibodies, but seropositive for anti-CCV and CRV antibody titers, were each administered a single oral dose of non-enteric coated vaccine. The dogs were bled once a week for 6 weeks and serum tested for anti-CPV, CCV and CRV antibodies. Response to vaccination was inconsistent (Table 1). Only two of the four dogs seroconverted to CPV (1/64 and 1/512, respectively, at 14 days post-vaccination; and 1/2,048 and 1/4,096, respectively, at 42 days post-vaccination). For both CCV and CRV, one out of four dogs showed an increase in titer following vaccination. The results were equivocal, possibly due to a failure to consistently deliver the antigen intact to the intestine.

TABLE 1

RESPONSE TO NON-ENTERIC COATED ORAL FORMULATION

Antigen	Serum Antibody Conversion/No. Dogs
CPV	2/4
CCV	1/4
CRV	1/4

EXAMPLE 3: EFFICACY TESTING OF AN ORAL FORMULATION WITH AN ENTERIC COATING

Attenuated CPV ($10^{7.7}$ $TCID_{50}$) and CCV ($10^{3.2}$ $TCID_{50}$) were formulated together into tablets as above in Example 2, but with an enteric coating, comprising about 3% (v/v) EUDRAGIT S100®, that dissolves only at about pH 6.0 or higher.

Twenty 7-week-old SPF beagles, seronegative for CPV and CCV were distributed into two groups of 10 dogs each, taking litter distribution into

consideration. The ten dogs in the first group (vaccinates) (Table 2) each received a single dose of the vaccine by placing a single tablet in the back of the mouth and gently pushing it into the throat for the dog to swallow. The ten dogs in the second group (controls) were not vaccinated (Table 3). Serum samples were collected prior to vaccination and for 5 weeks post-vaccination for CPV virus neutralization testing and CCV ELISA.

Since the dogs seroconverted to CCV prior to vaccination, indicating exposure to CCV, the efficacy of the CCV fraction of the vaccine could not be determined. However, the efficacy of the CPV fraction of the vaccine compared to the control was established, as shown in Tables 2 and 3. According to those results, the oral vaccine with the enteric coating elicited a rapid and strong protective immune response in all ten of the vaccinates (Table 2). All ten non-vaccinated control dogs failed to develop CPV serum neutralizing antibody titers (Table 3). This result is in contrast to that obtained with oral vaccine lacking an enteric coating, as demonstrated above in Example 2 (Table 1).

Observations were made regarding vaccine safety. Vaccinates were observed daily for 5 days post-vaccination for signs of vomiting, depression and diarrhea. In addition, temperatures were taken daily for 5 days post-vaccination, and blood collected once a week for CBC, acute-phase protein, and immune cell marker analyses. None of the vaccinates showed any signs of vomiting, diarrhea, depression, or a febrile response. Furthermore, none of the vaccinates showed any alpha-1 glycoprotein abnormalities, abnormal immune cell marker profiles, or abnormal blood profiles. Thus, the vaccine appears to be safe.

TABLE 2

CPV SERUM NEUTRALIZATION TITERS OF VACCINATED DOGS LACKING MATERNAL ANTI-CPV ANTIBODIES*

Vaccinate	Weeks Post-Vaccination					
Dog ID #	0	1	2	3	4	5
5001	<2	32	256	512	1,024	4,096
5007	<2	64	256	4,096	>4,096	2,048
5102	<2	32	256	512	2,048	2,048
5107	<2	32	512	1,024	2,048	2,048
5202	<2	2	1,024	512	2,048	2,048
5203	<2	<2	256	512	2,048	2,048
5302	<2	32	512	1,024	1,024	2,048
5401	<2	32	128	512	2,048	1,024
5502	<2	256	256	512	2,048	1,024
56013	<2	64	256	128	512	512
GMT[a]	**<2**	**24**	**315**	**630**	**1,663**	**1,663**

* = A serum neutralizing antibody titer equal to or greater than 1:16 is considered sufficient to protect against virulent CPV challenge (see 9 C.F.R. § 113.317).

a = Geometrical mean titer.

TABLE 3

CPV SERUM NEUTRALIZATION TITERS OF CONTROL DOGS

Control	Weeks Post-Vaccination					
Dog ID #	0	1	2	3	4	5
5002	<2	2	<2	<2	<2	<2
5005	<2	<2	<2	<2	<2	<2
5106	2	<2	<2	<2	<2	2
5201	<2	<2	<2	2	2	<2
5303	<2	2	<2	2	<2	<2
5304	<2	<2	<2	<2	<2	≤4
5403	<2	<2	<2	<2	<2	<2
5501	<2	<2	<2	<2	<2	<2
5504	<2	<2	<2	<2	<2	<2
5602	<2	<2	<2	<2	<2	<2
GMT	**<2**	**<2**	**<2**	**<2**	**<2**	**<2**

EXAMPLE 4: RESPONSE OF DOGS WITH MATERNAL ANTI-CPV ANTIBODIES TO ORAL CPV VACCINATION

Twenty 7-week-old beagles with maternal anti-CPV antibodies were divided into two groups, taking litter, sex and maternal antibody titer into consideration. Ten dogs in the first group (vaccinates) (Table 4) received one dose of the enteric-coated vaccine prepared as above. Ten dogs in the second group (controls) (Table 5) received no vaccine. A single tablet was placed in the back of the mouth of each vaccinate, and gently pushed into the throat for the dogs to swallow.

The data in Tables 4 and 5 demonstrate that the enteric-coated oral vaccine formulation of the invention immunizes dogs of weaning age against CPV infection in the presence of maternal anti-CPV antibody titers of up to 1:128. Out of four dogs with interfering maternal antibody titers of 1:128 (BGZ, BKY, BLY, CYZ), three (BGZ, BKY, BLY) developed strong serum-neutralizing antibody titers after a single oral dose of the vaccine. A second dose may be required for dogs having interfering maternal antibody titers greater than 1:128.

None of the vaccinates showed a febrile response or any evidence of vomiting, depression or diarrhea post-vaccination, again indicating that the vaccine is safe.

TABLE 4

CPV SERUM NEUTRALIZATION TITERS OF VACCINATED DOGS HAVING MATERNAL ANTI-CPV ANTIBODIES

Vaccinate	Weeks Post-Vaccination					
Dog ID #	0	1	2	3	4	5
BGZ	128	64	512	1,024	>4,096	>4,096
BKY	128	64	512	1,024	2,048	>4,096
BLY	128	32	512	256	256	1,024
CGZ	512	256	64	64	16	8
CIY	1,024	256	128	64	32	16
CYZ	128	64	32	8	8	8
CZY	512	256	128	32	16	16
DUZ	1,024	256	256	128	32	32
DXZ	1,024	256	256	32	32	32
EAY	1,024	256	128	64	64	32
GMT	**388**	**137**	**181**	**97**	**79**	**79**

TABLE 5

CPV SERUM NEUTRALIZATION TITERS OF CONTROL DOGS HAVING MATERNAL ANTI-CPV ANTIBODY TITERS

Control	Weeks Post-Vaccination					
Dog ID #	0	1	2	3	4	5
BHZ	256	64	32	32	8	16
BIZ	128	32	32	8	4	8
CHZ	512	128	128	64	16	16
CJY	1,024	512	256	128	64	32
CKY	256	128	64	64	32	16
DAY	256	64	64	64	8	16
DVZ	256	128	64	64	16	32
DWY	1,024	512	256	128	16	32
DYZ	256	256	32	16	8	8
EBY	512	256	256	128	64	32
GMT	**362**	**147**	**84**	**52**	**16**	**16**

All patents, patent applications, and publications cited above are incorporated herein by reference in their entirety.

The present invention is not to be limited in scope by the specific embodiments described, which are intended as single illustrations of individual aspects of the invention. Functionally equivalent compositions and methods are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the fields of immunology, veterinary medicine, delivery system formulations, and related fields, from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

I CLAIM:

1. An oral vaccine capable of triggering a protective immune response against a pathogen in an animal of weaning age or younger in the presence of interfering maternal antibodies, said vaccine comprising an immunologically effective amount of an antigen prepared from the pathogen in an enteric coating that dissolves only at about pH 6.0 or higher.

2. The oral vaccine of claim 1, wherein the antigen is prepared from a canine pathogen selected from the group consisting of canine parvovirus, canine distemper virus, canine coronavirus, and canine rotavirus.

3. The oral vaccine of claim 1, wherein the antigen is prepared from canine parvovirus.

4. The oral vaccine of claim 3, wherein the antigen is attenuated canine parvovirus.

5. The oral vaccine of claim 1, further comprising one or more excipients selected from the group consisting of lactose, sucrose, maltose, mannitol, starch, gum, gelatin, a cellulose derivative, magnesium stearate, magnesium carbonate, magnesium oxide, calcium carbonate, calcium sulfate, silica gel, and talc.

6. The oral vaccine of claim 1, further comprising lactose, magnesium stearate, modified cellulose gum, and an undercoating, and wherein the enteric coat comprises a partially methylated polymethacrylic acid copolymer.

7. The oral vaccine of claim 6, wherein the partially methylated polymethacrylic acid copolymer is EUDRAGIT S100® polymethacrylic acid copolymer, type B, NF.

8. A method for vaccinating an animal of weaning age or younger against a pathogen, comprising orally administering to the animal a vaccine capable of

triggering a protective immune response against the pathogen in the presence of interfering maternal antibodies, said vaccine comprising an immunologically effective amount of an antigen prepared from the pathogen in an enteric coating that dissolves only at about pH 6.0 or higher.

9. The method of claim 8, wherein the animal is a dog.

10. The method of claim 9, wherein the antigen is prepared from a pathogen selected from the group consisting of canine parvovirus, canine distemper virus, canine coronavirus, and canine rotavirus.

11. The method of claim 9, wherein the antigen is prepared from canine parvovirus.

12. The method of claim 11, wherein the antigen is attenuated canine parvovirus.

13. The method of claim 8, wherein the formulation further comprises one or more excipients selected from the group consisting of lactose, sucrose, maltose, mannitol, a gum, gelatin, a cellulose derivative, magnesium stearate, magnesium carbonate, magnesium oxide, calcium carbonate, calcium sulfate, silica gel, starch, and talc.

14. The method of claim 8, wherein the formulation further comprises lactose, magnesium stearate, modified cellulose gum, and an undercoating, and wherein the enteric coat comprises a partially methylated polymethacrylic acid copolymer.

15. The method of claim 14, wherein the partially methylated polymethacrylic acid copolymer is EUDRAGIT S100® polymethacrylic acid copolymer, type B, NF.

16. The method of claim 9, wherein the interfering maternal antibodies are present in the dog's serum in a titer of about 1:16 or higher.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 97/01136

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/28 A61K39/15 A61K39/175 A61K39/215 A61K39/23

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 152 413 A (GOODNOW ROBERT A) 1 May 1979 * col.2, l.44-45; col.4, l.2-25 & l.53-59; claims 10-12 *	1,5,8,13
Y	see the whole document	2-4,6,7, 9-12, 14-16

X	EP 0 181 117 A (AMERICAN HOME PROD) 14 May 1986 * claims 1,6,7 *	1,8
Y	see the whole document	2-7,9-16

Y	EP 0 420 459 A (WARNER LAMBERT CO) 3 April 1991 cited in the application * example; claims 1-11 *	1-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
15 January 1998	06. 02. 98

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 97/01136

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 9625 Derwent Publications Ltd., London, GB; Class B04, AN 96-249761 XP002052233 & RU 2 045 960 C (ULASOV V I) , 20 October 1995 see abstract -----	1-16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 97/01136

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4152413 A	01-05-79	DK 340079 A	19-02-80
		EP 0009577 A	16-04-80
		JP 55047617 A	04-04-80
EP 0181117 A	14-05-86	AU 576907 B	08-09-88
		AU 4884085 A	08-05-86
		CA 1263305 A	28-11-89
		DE 3586841 A	24-12-92
		DK 501785 A	02-05-86
		GB 2166349 A,B	08-05-86
		HK 5692 A	17-01-92
		IE 58205 B	28-07-93
		JP 2614996 B	28-05-97
		JP 8308575 A	26-11-96
		JP 2609447 B	14-05-97
		JP 61118326 A	05-06-86
		KR 9312116 B	24-12-93
		MX 9203610 A	01-07-92
		US 4920209 A	24-04-90
		US 5643579 A	01-07-97
EP 0420459 A	03-04-91	US 5032405 A	16-07-91
		AU 6314790 A	11-04-91
		CA 2026283 A	28-03-91
		JP 3184920 A	12-08-91